# Membrane organization in G-protein mechanisms

RICHARD R. NEUBIG[1]

Departments of Pharmacology and Internal Medicine (Hypertension), The University of Michigan, Ann Arbor, Michigan 48109-0626, USA

ABSTRACT **A prevailing view of receptor and G-protein function in cells includes random collisions between the proteins with a great specificity at the sites of protein-protein interaction. Recent evidence suggests that receptors, G-proteins, and effectors may be less mobile and that these systems are more highly organized than previously appreciated. Several types of evidence suggest that receptors do not have free access to all G-protein with which they are capable of coupling. Also, the specificity of signaling in intact cells appears to be significantly greater than in reconstituted systems. The distribution and mobility of G-proteins in cells are restricted to a surprising degree. Thus, complex interactions of the receptors and G-proteins with their effectors and cell membrane machinery appear to play an important role in their function. A full understanding of G-protein-coupled receptors must include a better description of the organization of these systems in cell membranes. Possible roles for noncoated pits (caveolae) and a novel pleckstrin homology domain need to be examined.—Neubig, R. R. Membrane organization in G-protein mechanisms. *FASEB J.* 8: 939-946; 1994.**

*Key Words: adrenergic receptor • diffusion • fluorescence • cytoskeleton • membrane fluidity • pleckstrin homology*

## SCOPE OF REVIEW

Signal transduction mediated by guanine nucleotide-binding proteins (G-proteins)[2] has been the focus of much interest since the early 1980s. These proteins play a central role in signal transduction and cell biology far beyond just activation of adenylyl cyclase (1-4). A tremendous amount is known about the multitude of different proteins involved in G-protein-coupled signal transduction (5-9). There are more than 200 receptor types and subtypes, at least 21 G-protein $\alpha$ subunits, 4 $\beta$ subunits, and 6 $\gamma$ subunits (10). There are at least six different subtypes of mammalian adenylyl cyclase (11), many phospholipases A and C (12), and numerous potassium and calcium channels that are regulated by G-proteins (3). In olfactory signaling there may even be an immunoglobulin-like diversity of receptor structure (13). How these diverse components are organized in the cell is an important area for future exploration. This review describes several types of evidence for a more complex organization of G-protein-coupled signal transducing molecules in cells and membranes than has been appreciated previously.

Much current thinking about G-protein-coupled receptors is based on the idea of freely mobile receptors, G-proteins, and effectors in which the specificity of their interactions derives solely from the three-dimensional structure of the sites of protein-protein interaction. This concept arose in part from the elegant studies by Orly and Schramm (14) showing that signal transducing components could be exchanged between cells. Additional support came from Tolkovsky and Levitzki (15) with their "collisional coupling model" and the demonstration of catalytic activation of adenylyl cyclase by $\beta$-adrenergic receptors. These ideas were further supported by studies of purified and reconstituted proteins (16-20) and the rhodopsin-transducin system (21). Although these approaches have yielded many useful insights into the mechanism of receptor action, rod outer segment disks provide an unusual membrane environment with a unique lipid composition (22). The lateral mobility of rhodopsin (translational diffusion coefficient, $D_T$, $5 \times 10^{-9}$ cm$^2$ • s$^{-1}$) is much higher than that of most mammalian membrane proteins (23). Receptors and G-proteins are also likely to be quite mobile in reconstituted lipid vesicles, making extrapolation to intact cells difficult.

A better understanding of the role of cytoskeleton in tyrosine kinase receptor signaling (24) raises the possibility of a specific organization of G-protein-coupled signal transducing molecules. The membranes of mammalian cells are far more complex than either reconstituted vesicles or rod outer segment membranes. Cells exhibit distinctive morphologies with functional proteins segregated into distinct regions (e.g., polarization of epithelial or neuronal cells), and even within relatively homogeneous regions there is an organized cytoskeletal network of proteins. Thus, it would not be surprising if receptor-G-protein-effector interactions in reconstituted systems differed in many ways from those in a complex plasma membrane or cell.

Recent evidence for more organized interactions of receptors, G-proteins, and effectors derives from several sources. First, several $G_i$-coupled receptors in neuronal cells appear to interact functionally with distinct "pools" of G-protein. Also, the existence of an uncoupled form of many $G_i$-coupled receptors is not compatible with free accessibility of homogeneous pools of receptors with excess G-protein. Second, the specificity of signaling by G-proteins and receptors in intact cells (25-27) appears to be greater than that seen in reconstitution systems (28, 29). Finally and most compellingly, direct demonstrations of G-protein-cytoskeleton interactions and limited mobility of receptors and G-proteins are becoming common. Indeed, there is recent recognition that

[1]Address correspondence and requests for reprints to Dr. Neubig at: Department of Pharmacology, University of Michigan M6322 Medical Science Bldg. 1, Ann Arbor, MI 48109-0626, USA.

[2]Abbreviations: $\beta$-ARK, $\beta$-adrenergic receptor kinase; $D_T$, translational diffusion coefficient; FPR, fluorescence photobleaching recovery; G-protein, guanine nucleotide binding protein; $G_i$, inhibitory G-protein; $G_s$, stimulatory G-protein; SH2, src homology 2; PH, pleckstrin homology domain; RG, receptor and G-protein complex; TMR, tetramethylrhodamine; TMR-$\beta\gamma$, tetramethylrhodamine-labeled $\beta\gamma$ subunit of G-protein; $I_m$, voltage-sensitive potassium current; LHRH, luteinizing hormone releasing hormone.

signal transduction events in intact cells are more complex and interesting than in reconstituted systems (9).

The multiplicity of receptors, G-proteins, and effectors (8, 11, 12, 30), structural features of the components (31, 32), mechanisms in reconstituted systems (33), and regulation (32) have been well reviewed recently. Thus, in this review I will focus on information regarding regulation of G-protein–receptor interactions in intact cells and membranes. I will describe accumulating data that support the idea of a higher-order organization of G-proteins and receptors in intact cells and membranes. Finally, I will speculate about possible cellular proteins that may play a role in this organization.

## CAN MULTIPLE RECEPTORS ACCESS THE SAME POOL OF G-PROTEINS IN CELLS OR CELL MEMBRANES?

### Evidence for shared pools of G-proteins

The nonadditivity of glucagon- and ACTH-stimulated adenylyl cyclase activity in adipocyte membranes showed that these receptors were both able to activate a common pool of effector enzyme (34). Additional evidence that multiple receptor types can access the same pool of G-protein comes from nonadditivity of receptor-stimulated GTPase activity. By this criterion, vasopressin and platelet-activating factor appear to share the same pool of G-protein in human platelet membranes (35). With agonist binding methods, Dasso and Taylor (36) showed that, in rat hepatocyte membranes, vasopressin was able to markedly reduce high-affinity binding of epinephrine to $\alpha_1$-adrenergic receptors apparently by tying up the G-protein required for the high-affinity conformation of the $\alpha_1$ receptor.

### Evidence for compartmentation of receptors and/or G-proteins

In other systems, there is evidence for compartmenation of receptors and/or G-proteins into distinct pools. This conclusion derives from functional data, ligand binding studies, and more direct physical evidence such as fluorescence photobleaching recovery measurements with G-protein subunits and coprecipitation with cytoskeletal proteins.

#### *Lack of cross-talk in agonist binding to $G_i$-coupled receptors*

In contrast to the results with vasopressin and $\alpha_1$-adrenergic receptors, $\alpha_2$-adrenergic and $\delta$-opioid receptors, both of which couple to the $G_{i2}$, showed no evidence for shared pools of G-protein in NG-108-15 neuroblastoma-glioma cells. Despite predictions of a two-receptor ternary complex model (37) there was no decrease in $\alpha_2$-adrenergic or $\delta$-opiate agonist binding by the heterologous agonist. This was true even when G-protein was made limiting by partial pertussis toxin treatment. This lack of cross-talk does not result from G-protein $\alpha$ subunit heterogeneity, as both the $\delta$ opiate and $\alpha_2$ receptor in NG 108-15 cells have been shown to efficiently couple to the $G_{i2}$ subtype of $G_i$; however, it could result from $\beta\gamma$ subunit heterogeneity (see below) or physical compartmentation of the G-protein.

#### *Agonist-specific GDP$\beta$S inhibition*

Another piece of data to indicate that multiple $G_i$-coupled receptors use distinct pools of $G_i$ comes from patch-clamp experiments with bullfrog sympathetic neurons (38). In this system, muscarinic agonists, substance P, and luteinizing hormone releasing hormone (LHRH) all inhibit a voltage-sensitive potassium current ($I_m$) through a pertussis toxin-sensitive $G_i$-like protein. Each agonist is capable of fully inhibiting the $I_m$ current in the cell and there is no additivity of responses. Inclusion of GDP$\beta$S in the pipette results in a time-dependent inhibition of responses that is accelerated by agonist. It is interesting that the effect is selective for the agonist applied with GDP$\beta$S. Treatment of cells with GDP$\beta$S, followed by several pulses of LHRH, causes a selective loss of the LHRH response whereas the muscarinic and substance P responses are spared. This is not just a receptor-desensitization phenomenon as it does not occur when GTP is in the pipette rather than GDP$\beta$S. The simplest explanation of these results is that agonist enhances the rate of GDP$\beta$S binding to the pool of G-protein that is coupled to that receptor. The fact that only the response to one receptor is blocked implies that the receptors do not share a common pool of G-protein; rather, each receptor seems to prefer its own pool of G-protein. This idea is a bit surprising because each receptor can fully activate the response (i.e., inhibition of $I_m$), but this can be rationalized by allowing an excess of G protein over effector and permitting the $\alpha$ subunits (which presumably mediate this effect (3)) to be mobile (**Fig. 1**).

#### *G-protein heterogeneity*

As the importance of $\beta$ and $\gamma$ subtypes becomes better understood, G-protein heterogeneity seems to play a much bigger role in receptor specificity than previously appreciated. Many different $\alpha$ and $\beta\gamma$ subunits have been identified (39, 40). Recent evidence of a strong selectivity of $\beta$ (26) and $\gamma$ (27) subunits for particular receptors may explain the limited G-protein pool available to receptors coupled to $G_i$-like G-proteins. For example, the selective interaction of somatostatin receptors with $\alpha_{o2}\beta_1\gamma_3$ and muscaninic receptors with $\alpha_{o1}\beta_3\gamma_4$, as shown by the data of Kleuss et al. (27), could account for the existence of G-protein "pools." This degree of selectivity, however, is not seen in reconstitution studies. This raises the interesting possibility that the $\beta\gamma$ subunits may impart their selectivity by localizing receptor, G-protein, and effector complexes in cell membranes rather than simply by imparting specificity at the receptor-$\beta\gamma$ interface.

#### *Restricted collision-coupling model*

Limited access of receptors to G-proteins is also supported by data on activation of adenylyl cyclase by adenosine $A_2$ receptors both in membranes and intact cells. Indeed, the original analysis by Braun and Levitzki (41) of adenosine receptor activation of adenylyl cyclase indicated that the adenosine receptor was more "tightly coupled" to the effector than was the $\beta$-adrenergic receptor. Gross and Lohse (42) describe a "restricted collision-coupling model" in which receptors diffuse freely but are only able to contact a small number of effectors. They do not, however, report how low the diffusion coefficient must be to account for their observations.

### Evidence for restricted mobility of receptors and/or G-proteins derived from ligand binding studies

A series of binding studies with the $\alpha_2$-adrenergic receptor led us to conclude that free diffusion was not the only factor regulating receptor–G-protein interactions in cell membranes (43–45). The first evidence for this conclusion was the existence of an uncoupled state of the $\alpha_2$-adrenergic receptor

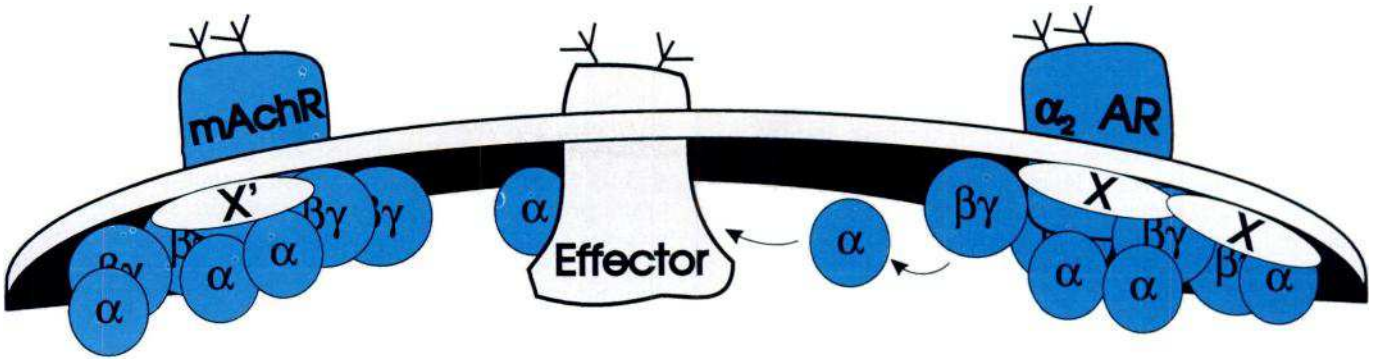


**Figure 1.** Possible membrane organization of receptors and G-proteins. The interactions of receptors and G-proteins do not appear to be governed entirely by a random collision of proteins in the plasma membrane. Rather, sorting of the distinct components of the G-protein-mediated signal transduction system into organized regions or supramolecular complexes may be important for the specificity of receptor-G-protein interactions in cells. Different receptors (e.g., $\alpha_2$ adrenergic and m4 muscarinic) that are capable of coupling to the same G-protein in vitro do not seem to share the same pool of G-protein in intact cells. It will be important to identify any additional cellular machinery (X or X') that may be responsible for the organization of these systems in cells. The interaction of one receptor with multiple $\beta\gamma$ subunits suggested in this model is consistent with the significant excess of G-proteins over receptors in most tissues. It is also possible that some effector molecules could be incorporated into the complexes of receptors and $\beta\gamma$ subunits as there is increasing evidence for signal transmission by $\beta\gamma$ subunits as well as $\alpha$ subunits of G-proteins.

in equilibrium binding studies despite a 20- to 50-fold excess of G-protein (43). Second, there was a slow agonist-stimulated association of the $\alpha_2$-adrenergic receptor and G-protein in kinetic studies that was more than an order of magnitude slower than that predicted for simple protein diffusion in lipid membranes (44, 45).

To appreciate the significance of low-affinity agonist binding in the presence of excess G-protein we must closely examine the predictions of the ternary complex model of ligand binding (46). Many investigators have pointed out that the ternary complex model only accounts for the biphasic agonist binding if the G-protein is present in amounts less than or equal to that of receptor (43, 46–48). We showed that in human platelet membranes there is a 20- to 50-fold excess of $G_i$ over $\alpha_2$-adrenergic receptor (43). This discrepancy made it clear that the simple ternary complex model was not sufficient to explain the existence of low-affinity binding. Ransnäs and Insel (50) quantitated $G_s$ in S49 lymphoma cells and also found a great excess of $G_s$ (19 pmol/mg) over $\beta$-adrenergic receptors (170 fmol/mg) in that system as well. There is also a significant excess of G-protein over receptor for formylpeptide (51), muscarinic (52), and opiate (52) receptors, which all show biphasic agonist binding.

Thus, for many $G_i$-coupled receptors and at least one $G_s$-coupled receptor, an explanation for the low-affinity binding was needed beyond the simple ternary complex model. There are two general types of explanations: *1)* biochemical heterogeneity of receptor or G-protein, and *2)* compartmentation of receptor and/or G-protein in cells to prevent access. It is possible that phosphorylation of receptors (53–55) could result in an uncoupled receptor with low affinity for agonist, but other sources of receptor heterogeneity including receptor subtypes, alternative splicing of mRNA, glycosylation, and palmitoylation do not account for the many systems in which low-affinity binding is seen. The $\alpha$ subunits do not exhibit enough heterogeneity to result in a limiting amount of G-protein for coupling to $\alpha_2$- and $\beta$-adrenergic or neutrophil formylpeptide receptors.[3] A further level of heterogeneity may also be present in the interactions of receptors with coated pits and noncoated membrane invaginations (caveolae). This is discussed later in this review under the rubric of compartmentation.

Detailed agonist binding time course studies have been described for the $\alpha_2$-adrenergic receptor (44, 45, 57) and the neutrophil formylpeptide receptor (58–60). In both systems, there appear to be three states of the receptor: *1)* the low-affinity resting state of the receptor (R), which couples to G-protein after agonist is added, *2)* a precoupled complex of receptor and G-protein (RG) with high affinity for agonist, and *3)* an "uncouplable" state of the receptor (R') that is never able to couple to the G protein and has low affinity for agonist.

Approximately one-third to one-half of the receptors appear to be in the precoupled state for both receptors. Upon addition of agonist, slow coupling of an additional 30–50% of the receptor occurs with a half-time of 300 to 700 s while the predicted value for simple diffusion with a $D_T$ of $5 \times 10^{-10}$ cm$^2 \cdot$ s$^{-1}$ is less than a second (44).

## "Structural" studies

More direct methods also indicate that the simple concept of receptors and G-proteins moving freely in cell membranes needs to be modified. Indeed, the existence of cytoplasmic barriers to free motion of proteins is well supported (61).

### *Target size analysis*

Early work on the glucagon receptor-$G_s$-adenylyl cyclase system from Rodbell and co-workers (62) found that target sizes determined from radiation inactivation studies did not fit with a simple idea of receptor and G-protein monomers

[3] In the case of the $\alpha_2$-adrenergic receptor, there is a 50-fold excess of $G_i$ over receptor in human platelet membranes (43) and the $\alpha_{i2}$ subunit makes up approximately two-thirds of the total $G_i$ pool (39), which still leaves a 30-fold excess of $G\alpha_{i2}$ protein that should be able to couple to $\alpha_2$ receptors. An estimate of the number of $G_i$ and receptor molecules in each platelet membrane fragment is 120 and 2.4, respectively (44), which if evenly distributed would result in an infinitesimal fraction of the membrane fragments with no G-protein. Thus, only a highly compartmentalized distribution of G-protein could result in membrane vesicles that contain receptor but no G-protein.

floating freely in cell membranes. Large complexes with predicted molecular weights in excess of 1 million were found. In addition, Nakamura and Rodbell (63) found that extraction of hepatic cell membranes with the detergent octylglucoside resulted in large complexes containing the $G_s$ $\alpha$ subunits. The size of these complexes was reduced by previous treatment of the cells with glucagon (64). The implications of these data have been reviewed recently and a model involving large macromolecular assemblies of G-protein was proposed (9).

*Cytoskeletal associations*

A number of laboratories have obtained data showing association of G-protein $\alpha$ or $\beta\gamma$ subunits with tubulin or other cytoskeletal proteins. An interaction between tubulin and adenylyl cyclase was suggested from effects of microtubule inhibitors on adenylyl cyclase activity (see review, ref 65). More direct evidence for interactions of G-proteins with microtubules came from transfer of guanine nucleotides from G-proteins to tubulin (66) and direct binding of $^{125}$I-labeled tubulin to G-protein $\alpha$ subunits, particularly $G_{i1}$ and $G_s$ (67, 68). $G_i$-like proteins in a murine T lymphoma cell line colocalize in cap structures that are rich in actin, myosin, and fodrin (69). Also, in S49 lymphoma cells the $\beta\gamma$ subunit of G-proteins appears in a Triton X-100 insoluble pool that is enriched in actin (70). Finally, the formylpeptide receptor in neutrophils appears to be intimately involved in cytoskeletal interactions (71, 72). The possible role of spectrin or dynamin in binding $\beta\gamma$ subunits is discussed below with respect to pleckstrin homology domains.

*Immunocytochemical localization of receptors and G-proteins*

In epithelial tissues and in neurons, endogenous G-proteins (**Table 1**) and epitope-tagged adrenergic receptors (73, 74) both show markedly specific distributions within cells. By immunocytochemical methods, $G_i$ and $G_o$ both exhibit a clustered distribution in the plasma membrane and may be associated with microspikes enriched in actin (75, 76) (Table 1). $G_o$ is also localized in growth cones of neurons (28). The cellular distribution of the $\beta$-adrenergic receptor in A431 cells exhibits a striking speckled pattern indicating a nonrandom distribution of the receptors in the plasma membrane (77). Immunofluorescence localization of G-protein $\beta$ subunits in these cells (77) and in transfected COS cells (78) also showed a speckled pattern. These observations all suggest that a specific mechanism is responsible for the organization of components of the signal transduction cascade in cell membranes.

*Fluorescence photobleaching recovery measurements with G-protein subunits*

Despite the important role of the collisional-coupling model (15, 79) in the theory of G-protein-coupled receptors, there has been virtually no direct data on the mobility of G-proteins in membranes or intact cells (80). There are only two systems for which mobility has been studied for G-protein-coupled receptors (81, 82). We found that fluorescently labeled G-protein $\alpha$ and $\beta\gamma$ subunits (83) introduced into NG 108-15 neuroblastoma-glioma cells (83) by cell-vesicle fusion showed a strong degree of clustering, similar to that reported for immunofluorescence studies of endogenous G-proteins (see above). Although the labeled $\beta\gamma$ subunit was largely located in the plasma membrane, the $\alpha$ subunit showed both membrane and cytoplasmic distributions (83). Most strikingly, the mobility of the tetramethylrhodamine-labeled $\beta\gamma$ (TMR-$\beta\gamma$) subunit of $G_o$ in NG cells as determined by fluorescence photobleaching recovery (FPR) was very low. Only 16% of the fluorescence of TMR-$\beta\gamma$ recovered after photobleaching (83). The small fraction that did recover had a diffusion coefficient of $2 \times 10^{-9}$ cm$^2 \cdot$s$^{-1}$, slightly higher than that of typical mammalian cell membrane proteins. TMR-labeled $\alpha_o$ subunits also showed less recovery of fluorescence than expected (32% vs. 50–60% for typical membrane proteins) and had a slightly higher diffusion coefficient than $\beta\gamma$ ($4 \times 10^{-9}$ cm$^2 \cdot$s$^{-1}$). Thus, the restricted mobility of components of the G-protein-coupled signal transduction cascade does not appear to be due to a uniformly low diffusion coefficient; rather, a large fraction of $\beta\gamma$ subunit is virtually immobilized.

No effect on either the mobile fraction or diffusion coefficients of the $\alpha$ or $\beta\gamma$ subunits was seen upon addition of agonist or modification of cytoskeletal proteins with cytochalasin or nocodozole (83). The difference between the mobility of the $\alpha$ and $\beta\gamma$ is intriguing. It has been proposed that the $\beta\gamma$ subunits are a membrane anchor for the $\alpha$ subunit (84, 85) and the $\beta$ subunit has been shown to associate with cytoskeleton (70). Consistent with this concept, fluorescence energy transfer studies show that fluorescent labels in $\beta$ and $\gamma$ subunits are closer to membrane lipids than the label in $\alpha$ subunit (86). Future work should focus on the specificity of $\beta\gamma$ subunit anchoring rather than just considering it a property of the "hydrophobic" nature of the $\beta\gamma$ subunit.

*Molecular candidates for organizing factors*

Two recent observations provide interesting speculations about possible cellular or molecular candidates for regulating movement of G-proteins in cell membranes. First, a striking enrichment of several types of G-proteins was found in Triton X-100 insoluble membranes with many characteristics of noncoated pits or caveolae (87). These membranes were also enriched in caveolin, the protein component of noncoated pits that binds glycosylphosphatidylinositol-linked proteins and plays a major role in protein sorting in epithelial cells. $\beta$-Adrenergic receptors were also shown to accumulate in noncoated pits (88) and to copurify with caveolin (89) when cross-linked with antibodies. It is interesting that both calcium ATPase and calcium release channels (inositol trisphosphate receptor) are also enriched in caveolae (90, 91). Thus, caveolae may represent sites of assembly of a signal transducing complex that could include receptors, G-proteins, effectors, and even the intracellular targets of the second messengers generated.

Second, at a molecular level the novel protein interaction motif called the pleckstrin homology (PH) domain (92, 93) may be important for $\beta\gamma$ subunit interactions with the cytoskeleton and other cellular proteins. The PH domain was named for pleckstrin, the major protein kinase C substrate in human platelets, and is similar to src homology 2 (SH2) domains. PH domains have been found in signal transducing molecules such as ras-GTPase activating protein and ras-guanine nucleotide releasing factor (93). They are also found in the cytoskeletal protein spectrin and the microtubule motor protein dynamin (92). It was recently observed that the carboxyl-terminal portion of $\beta$-ARK, which is the site of interaction with $\beta\gamma$ subunits (94), also contains a PH domain (95, 96). This region is missing from rhodopsin kinase, which is not activated by $\beta\gamma$ subunits. This provides an intriguing clue that PH domains are important for $\beta\gamma$ subunit interactions with other proteins. It leads to the

TABLE 1. *Localization and subcellular distribution of G-protein subunits*[a]

| Component | Method | Cell type | Result | Reference |
|---|---|---|---|---|
| | | **Cells** | | |
| $\alpha_s$, $\alpha_i$ | SCF | Adipocytes | *1)* $\alpha$ subunits eightfold enriched in PM compared with low-density membranes (Golgi)<br>*2)* $\beta\gamma$ not detected in low-density membranes (Golgi) | (97) |
| $\alpha_s$, $\alpha_i$, $\alpha_o$ | IEM | Cardiomyocytes | All labeling in PM, no staining of sarcoplasmic reticulum | (98) |
| $\alpha_i$ | IC<br>IEM | 3T3 cells, NRKF, C6 glioma, HUVE HEK293 | *1)* Cytoplasmic tubular structures were stained (mitochondria or microtubules)<br>*2)* Diffuse particulate fluorescence in PM<br>*3)* Concentrated staining of PM in microspikes | (76) |
| $\alpha_{i3}$ | IC | Transfected NIH-3T3 cells | *1)* Low $\alpha$ subunit densities—Golgi<br>*2)* High expression densities—Golgi and PM | (99) |
| $\alpha_{i3}$ | IC and SCF | $LLPK_1$, NRK, and rat liver and transfected $LLPK_1$ | $\alpha_{i3}$ present in Golgi | (100) |
| $\beta\gamma$ | IC and SCF | Transfected COS cells | *1)* $\beta\gamma$ shows punctate distribution in PM but is also present in intracellular vesicles<br>*2)* Inhibition of $\gamma$ subunit prenylation results in substantial cytoplasmic pool of $\beta\gamma$ | (78) |
| | | **Tissues** | | |
| $\alpha_{i3}$ | IC | A6 renal epithelial cells | *1)* $\alpha_{i3}$ and sodium channels in "distinct but adjacent domains of the apical cell surface"<br>*2)* Only $\alpha_{i3}$ in Golgi, no sodium channels | (101) |
| $\alpha_s$, $\alpha_i$, $\beta\gamma$ | SCF<br>IC | Kidney | *1)* Cortex—$\alpha_s$ and $\alpha_i$ greater in brush border vs. basolateral membrane<br>*2)* Medulla—$\alpha_s$ in both brush border and basolateral membrane but $\alpha_{i2}$ only in brush border | (102) |
| $\alpha_s$, $\alpha_{i1,2,3}$ | IC | Rat kidney | *1)* $\alpha_{i1}$ apical pole<br>*2)* $\alpha_{i2}$ basolateral PM and cytoplasm of collecting duct cells<br>*3)* $\alpha_{i3}$ apical proximal tubule "sub-brush border invaginations" and perinuclear Golgi<br>*4)* $\alpha_s$ basolateral PM of thick ascending limb cells | (103) |
| $\alpha$ and $\beta\gamma$ | IC and SCF | *Aplysia* nervous tissue | $\alpha$ and $\beta\gamma$ both in PM fraction of syaptosomes, not granules | (104) |
| $\alpha_i$ and $\alpha_o$ $\beta\gamma$ | IC | Olfactory tissue | *1)* Epithelium—$\alpha_o$, $\alpha_{i2}$, and $\beta\gamma$ all present in basolateral surface but apical surface has $\beta\gamma$ only<br>*2)* Molecular and internal granular layer of olfactory bulb has $\alpha_o$ and $\alpha_{i1/3}$ present but no $\alpha_{i2}$ | (105) |
| $\alpha_{i2}$ | IC, SCF, and IEM | Brain | *1)* $\alpha_{i2}$ in axon terminals<br>2) $\alpha_{i2}$ in PM, ER, synaptic vesicles, and microtubules | (106) |

[a]Studies from 1990-1993 of the subcellular distribution and immunocytochemical localization of G-protein subunits in cells and tissues reveal restricted localizations of different subunits within and between cells. IC, immunocytochemistry; SCF, subcellular fractionation; IEM, immunoelectron microscopy; PM, plasma membrane.

speculation that the PH domains in cytoskeletal proteins such as spectrin or dynamin may bind $\beta\gamma$ subunits and contribute to their immobility and organization in cell membranes.

## SUMMARY

A wide variety of approaches have yielded data that are not consistent with the simple concept that G-proteins and their coupled receptors move freely in cells. This raises the possibility that the interaction of G-proteins and receptors with one another may not be based solely on the sequence of the interaction sites on the proteins themselves but depends on their organization in the cell. Rodbell (9) has proposed the idea that G-protein activation involves multimers of G-proteins that exhibit a dynamic instability like actin. The concept proposed here incorporates his observations of large molecular complexes containing G-proteins and our data on the highly restricted mobility of the G-protein $\beta\gamma$ subunits in cells. Thus, there appears to be a more explicit organization of receptors, G-proteins, and probably effectors into supramolecular complexes that enhance the specificity of the protein-protein interactions (Fig. 1). Are there other components of the cell membrane and/or cytoskeleton (i.e., X or X' in Fig. 1) that limit the mobility of $\alpha$ and $\beta\gamma$ subunits of G-proteins? If so, are they spectrin- or dynamin-like proteins? Do they also organize the receptors and G-proteins in clusters? Is this organization specific for the type of receptor and G-protein? Is the role of the $\beta\gamma$ subunit subtype in specificity of receptor coupling (26, 27) due to a direct inter-

action of $\beta\gamma$ subunit with the receptor or is it due to a targeting of that $\beta\gamma$ subunit to the vicinity of the receptor? It is clear that the simple model of receptors and G-proteins floating in a Singer-Nicolson (107) sea of lipid needs to be reevaluated and more specific questions asked regarding the formation and maintenance of a sophisticated level of cellular organization of these important signal transducing components. FJ

The author thanks Drs. Ann Remmers and Bill Pratt for a critical review of the manuscript, Dr. Staffan Uhlén for helpful discussions, and Dr. Henry Bourne for many helpful comments. Support for the work described in this review was from the National Institutes of Health (GM 39561) and the author is an American Heart Association/Genentech Inc. Established Investigator.

*Note added in proof:* Two recent papers (Lisanti et al., *Trends Cell. Biol.*, vol. 4, pp. 231–235, 1994, and Touhova et al., *J. Biol. Chem.*, vol. 269, pp. 10217–10220, 1994) discuss the role of caveolae and PH domains, respectively, in G-protein function.

## REFERENCES


1. Limbird, L. E. (1988) Receptors linked to inhibition of adenylate cyclase: additional signaling mechanisms. *FASEB J.* **2,** 2686–2695
2. Freissmuth, M., Casey, P. J., and Gilman, A. G. (1989) G proteins control diverse pathways of transmembrane signalling. *FASEB J.* **3,** 2125–2131
3. Brown, A. M. (1991) A cellular logic for G protein-coupled ion channel pathways. *FASEB J.* **5,** 2175–2179
4. Gupta, S. K., Gallego, C., and Johnson, G. L. (1992) Mitogenic pathways regulated by G protein oncogenes. *Mol. Biol. Cell* **3,** 123–128
5. Gilman, A. G. (1987) G proteins: transducers of receptor-generated signals. *Annu. Rev. Biochem.* **56,** 615–649
6. O'Dowd, B. F., Lefkowitz, R. J., and Caron, M. G. (1989) Structure of the adrenergic and related receptors. *Annu. Rev. Neurosci.* **12,** 67–83
7. Dohlman, H. G., Caron, M. G., and Lefkowitz, R. J. (1987) A family of receptors coupled to guanine nucleotide regulatory proteins. *Biochemistry* **26,** 2657–2664
8. Simon, M. I., Strathmann, M. P., and Gautam, N. (1991) Diversity of G proteins in signal transduction. *Science* **252,** 802–808
9. Rodbell, M. (1992) The role of GTP-binding proteins in signal transduction: from the sublimely simple to the conceptually complex. *Curr. Top. Cell. Regul.* **32,** 1–47
10. Hepler, J. R., and Gilman, A. G. (1992) G proteins. *Trends Biochem. Sci.* **17,** 383–387
11. Tang, W. J., and Gilman, A. G. (1992) Adenylyl cyclases. *Cell* **70,** 869–872
12. Rhee, S. G., and Choi, K. D. (1992) Multiple forms of phospholipase C isozymes and their activation mechanisms. *Adv. Second Messenger Phosphoprotein Res.* **26,** 35–61
13. Buck, L., and Axel, R. (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* **65,** 175–187
14. Orly, J., and Schramm, M. (1976) Coupling of catecholamine receptor from one cell with adenylate cyclase from another cell by cell fusion. *Proc. Natl. Acad. Sci. USA* **73,** 4410–4414
15. Tolkovsky, A. M., and Levitzki, A. (1978) Mode of coupling between the $\beta$-adrenergic receptor and adenylate cyclase in turkey erythrocytes. *Biochemistry* **17,** 3795–3810
16. Asano, T., and Ross, E. M. (1984) Catecholamine-stimulated guanosine 5'-O(3-thiophosphate) binding to the stimulatory GTP-binding protein of adenylate cyclase: kinetic analysis in reconstituted phospholipid vesicles. *Biochemistry* **23,** 5467–5471
17. Cerione, R. A., Codina, J., Benovic, J. L., Lefkowitz, R. J., Birnbaumer, L., and Caron, M. G. (1984) The mammalian $\beta_2$-adrenergic receptor: reconstitution of functional interactions between pure receptor and pure stimulatory nucleotide binding protein of the adenylate cyclase system. *Biochemistry* **23,** 4519–4525
18. May, D. C., Ross, E. M., Gilman, A. G., and Smigel, M. D. (1985) Reconstitution of catecholamine-stimulated adenylate cyclase activity using three purified proteins. *J. Biol. Chem.* **260,** 15829–15833
19. Levitzki, A. (1985) Reconstitution of membrane receptor systems. *Biochim. Biophys. Acta* **822,** 127–153
20. Cerione, R. A. (1991) Reconstitution of receptor/GTP-binding protein interactions. *Biochim. Biophys. Acta* **1071,** 473–501
21. Chabre, M., and Deterre, P. (1989) Molecular mechanism of visual transduction. *Eur. J. Biochem.* **179,** 255–266
22. Stone, W. L., Farnsworth, C. C., and Dratz, E. A. (1979) A reinvestigation of the fatty acid content of bovine, rat and frog retinal rod outer segments. *Exp. Eye Res.* **28,** 387–397
23. Axelrod, D. (1983) Lateral motion of membrane protein and biological function. *J. Membr. Biol.* **75,** 1–10
24. Schaller, M. D., and Parsons, J. T. (1993) Focal adhesion kinase: an integrin-linked protein tyrosine kinase. *Trends Cell. Biol.* **3,** 258–262
25. Kleuss, C., Hescheler, J., Ewel, C., Rosenthal, W., Schultz, G., and Wittig, B. (1991) Assignment of G-protein subtypes to specific receptors inducing inhibition of calcium currents. *Nature (London)* **353,** 43–48
26. Kleuss, C., Scherubl, H., Hescheler, J., Schultz, G., and Wittig, B. (1992) Different $\beta$-subunits determine G-protein interactions with transmembrane receptors. *Nature (London)* **358,** 424–426
27. Kleuss, C., Scherubl, H., Hescheler, J., Schultz, G., and Wittig, B. (1993) Selectivity in signal transduction determined by gamma subunits of heterotrimeric G proteins. *Science* **259,** 832–834
28. Rubenstein, R. C., Linder, M. E., and Ross, E. M. (1991) Selectivity of the beta-adrenergic receptor among $G_s$, $G_i$'s, and $G_o$: assay using recombinant alpha subunits in reconstituted phospholipid vesicles. *Biochemistry* **30,** 10769–10777
29. Kurose, H., Regan, J. W., Caron, M. G., and Lefkowitz, R. J. (1991) Functional interactions of recombinant alpha 2 adrenergic receptor subtypes and G proteins in reconstituted phospholipid vesicles. *Biochemistry* **30,** 3335–3341
30. Bourne, H. R., Sanders, D. A., and McCormick, F. (1991) The GTPase superfamily: conserved structure and molecular mechanism. *Nature (London)* **349,** 117–127
31. Conklin, B. R., and Bourne, H. R. (1993) Structural elements of $G\alpha$ subunits that interact with $G\beta\gamma$, receptors, and effectors. *Cell* **73,** 631–641
32. Caron, M. G., and Lefkowitz, R. J. (1993) Catecholamine receptors: structure, function, and regulation. *Recent Prog. Horm. Res.* **48,** 277–290
33. Cerione, R. A., and Ross, E. M. (1991) Reconstitution of receptors and G proteins in phospholipid vesicles. *Methods Enzymol.* **195,** 329–342
34. Birnbaumer, L., Pohl, S. L., Michiel, H., Krans, M. J., and Rodbell, M. (1970) The actions of hormones on the adenyl cyclase system. *Adv. Biochem. Psychopharmacol.* **3,** 185–208
35. Houslay, M. D., Bojanic, D., Gawler, D., O'Hagan, S., and Wilson, A. (1986) Thrombin, unlike vasopressin, appears to stimulate two distinct guanine nucleotide regulatory proteins in human platelets. *Biochem. J.* **238,** 109–113
36. Dasso, L. L. T., and Taylor, C. W. (1992) Different calcium-mobilizing receptors share the same guanine nucleotide-binding protein pool in hepatocytes. *Mol. Pharmacol.* **42,** 453–457
37. Graeser, D., and Neubig, R. R. (1993) Compartmentation of receptors and G proteins in NG108-15 cells: lack of cross-talk in agonist binding among the alpha2 adrenergic, muscarinic and opiate receptors. *Mol. Pharmacol.* **43,** 434–443
38. Simmons, M. A., and Mather, R. J. (1991) Selectivity of the effects of guanosine-5'-O-($\gamma$-thiodiphosphate) on agonist inhibition of the M-current in amphibian sympathetic neurons. *J. Neurosci.* **11,** 2130–2134
39. Simonds, W. F., Goldsmith, P. K., Codina, J., Unson, C. G., and Spiegel, A. M. (1989) $G_{i2}$ mediates $\alpha_2$-adrenergic inhibition of adenylate cyclase in platelet membranes: in situ identification with $G\alpha$ C-terminal antibodies. *Proc. Natl. Acad. Sci. USA* **86,** 7809–7813
40. Gerhardt, M. A., and Neubig, R. R. (1991) Multiple $G_i$ subtypes couple to a single effector mechanism. *Mol. Pharmacol.* **40,** 707–711
41. Braun, S., and Levitzki, A. (1979) Adenosine receptor permanently coupled to turkey erythrocyte adenylate cyclase. *Biochemistry* **18,** 2134–2138
42. Gross, W., and Lohse, M. J. (1991) Mechanism of activation of $A_2$ adenosine receptors. II. A restricted collision-coupling model of receptor-effector interaction. *Mol. Pharmacol.* **39,** 524–530
43. Neubig, R. R., Gantzos, R. D., and Brasier, R. S. (1985) Agonist and antagonist binding to $\alpha_2$-adrenergic receptors in purified membranes from human platelets: implications of receptor-inhibitory nucleotide binding protein stoichiometry. *Mol. Pharmacol.* **28,** 475–486
44. Neubig, R. R., Gantzos, R. D., and Thomsen, W. J. (1988) Mechanism of agonist and antagonist binding to $\alpha_2$ adrenergic receptors: evidence for a precoupled receptor-guanine nucleotide protein complex. *Biochemistry* **27,** 2374–2384
45. Gantzos, R. D., and Neubig, R. R. (1988) Temperature effects on $\alpha_2$-adrenergic receptor-$G_i$ interactions. *Biochem. Pharmacol.* **37,** 2815–2821
46. De Lean, A., Stadel, J. M., and Lefkowitz, R. J. (1980) A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled $\beta$-adrenergic receptor. *J. Biol. Chem.* **255,** 7108–7117
47. Wreggett, K. A., and De Lean, A. (1984) The ternary complex model. Its properties and application to ligand interactions with the $D_2$-dopamine receptor of the anterior pituitary gland. *Mol. Pharmacol.* **26,** 214–227

48. Wong, H. M., Sole, M. J., and Wells, J. W. (1986) Assessment of mechanistic proposals for the binding of agonists to cardiac muscarinic receptors. *Biochemistry* **25**, 6995-7008
49. Deleted in proof
50. Ransnäs, L. A., and Insel, P. A. (1988) Quantitation of the guanine nucleotide binding regulatory protein $G_s$ in S49 cell membranes using antipeptide antibodies to $\alpha_s$. *J. Biol. Chem.* **263**, 9482-9485
51. Mueller, H., Weingarten, R., Ransnäs, L. A., Bokoch, G. M., and Sklar, L. A. (1991) Differential amplification of antagonistic receptor pathways in neutrophils. *J. Biol. Chem.* **266**, 12939-12943
52. Asano, T., Morishita, R., Sano, M., and Kato, K. (1989) The GTP-binding proteins, $G_o$ and $G_{i2}$, of neural cloned cells and their changes during differentiation. *J. Neurochem.* **53**, 1195-1198
53. Lefkowitz, R. J., and Caron, M. G. (1986) Regulation of adrenergic receptor function by phosphorylation. *Curr. Top. Cell Regul.* **28**, 209-231
54. Sibley, D. R., Benovic, J. L., Caron, M. G., and Lefkowitz, R. J. (1987) Regulation of transmembrane signaling by receptor phosphorylation. *Cell* **48**, 913-922
55. Sibley, D. R., Strasser, R. H., Benovic, J. L., Daniel, K., and Lefkowitz, R. J. (1986) Phosphorylation/dephosphorylation of the beta-adrenergic receptor regulates its functional coupling to adenylate cyclase and subcellular distribution. *Proc. Natl. Acad. Sci. USA* **83**, 9408-9412
56. Deleted in proof.
$\alpha_2$ agonist induced receptor-$N_i$ coupling. *Federation Proc.* **46**, 702 (abstr.)
57. Neubig, R. R., and Thomsen, W. J. (1987) Rapid kinetics of alpha$_2$ adrenergic agonist binding. In *Membrane Proteins: Proceedings of a Symposium* (Goheen, S. C., ed) pp. 619-632, Bio-Rad Laboratories, Richmond, California
58. Sklar, L. A., Mueller, H., Omann, G., and Oades, Z. (1989) Three states for the formyl peptide receptor on intact cells. *J. Biol. Chem.* **264**, 8483-8486
59. Fay, S. P., Posner, R. G., Swann, W. N., and Sklar, L. A. (1991) Real-time analysis of the assembly of ligand, receptor, and G protein by quantitative fluorescence flow cytometry. *Biochemistry* **30**, 5066-5075
60. Posner, R. G., Fay, S. P., Domalewski, M. D., and Sklar, L. A. (1994) Continuous spectral fluorometric analysis of formyl peptide receptor ternary complex interaction. *Mol. Pharmacol.* **45**, 65-73
61. Edidin, M., Kuo, S. C., and Sheetz, M. P. (1991) Lateral movements of membrane glycoproteins restricted by dynamic cytoplasmic barriers. *Science* **254**, 1379-1382
62. Schlegel, W., Kempner, E. S., and Rodbell, M. (1979) Activation of adenylate cyclase in hepatic membranes involves interactions of the catalytic unit with multimeric complexes of regulatory proteins. *J. Biol. Chem.* **254**, 5168-5176
63. Nakamura, S.-I., and Rodbell, M. (1990) Octyl glucoside extracts GTP-binding regulatory proteins from rat brain "synaptoneurosomes" as large, polydisperse structures devoid of $\beta\gamma$ complexes and sensitive to disaggregation by guanine nucleotides. *Proc. Natl. Acad. Sci. USA* **87**, 6413-6417
64. Nakamura, S.-I., and Rodbell, M. (1991) Glucagon induces disaggregation of polymer-like structures of the $\alpha$ subunit of the stimulatory G protein in liver membranes. *Proc. Natl. Acad. Sci. USA* **88**, 7150-7154
65. Rasenick, M. M., O'Callahan, C. M., Moore, C. A., and Kaplan, R. S. (1985) GTP-binding proteins which regulate neuronal adenylate cyclase interact with microtubule proteins. In *Microtubules and Microtubule Inhibitors* (DeBrabander, M., and DeMey, J., eds) pp. 313-325, Elsevier, Amsterdam
66. Rasenick, M. M., and Wang, N. (1988) Exchange of guanine nucleotides between tubulin and GTP-binding proteins that regulate adenylate cyclase: cytoskeletal modification of neuronal signal transduction. *J. Neurochem.* **51**, 300-311
67. Rasenick, M. M., Wang, N., and Yan, K. (1990) Specific associations between tubulin and G proteins: participation of cytoskeletal elements in cellular signal transduction. *Adv. Second Messenger Phosphoprotein Res.* **24**, 381-386
68. Wang, N., and Rasenick, M. M. (1991) Tubulin-G protein interactions involve microtubule polymerization domains. *Biochemistry* **30**, 10957-10965
69. Bourguignon, L. Y. W., Walker, G., and Huang, H. S. (1990) Interactions between a lymphoma membrane-associated guanosine 5'-triphosphate-binding protein and the cytoskeleton during receptor patching and capping. *J. Immunol.* **144**, 2242-2252
70. Carlson, K. E., Woolkalis, M. J., Newhouse, M. G., and Manning, D. R. (1986) Fractionation of the beta subunit common to guanine nucleotide-binding regulatory proteins with the cytoskeleton. *Mol. Pharmacol.* **30**, 463-468
71. Jesaitis, A. J., Bokoch, G. M., Tolley, J. O., and Allen, R. A. (1988) Lateral segregation of neutrophil chemotactic receptors into actin- and fodrin-rich plasma membrane microdomains depleted in guanyl nucleotide regulatory proteins. *J. Cell Biol.* **107**, 921-928
72. Sarndahl, E., Lindroth, M., Bengtsson, T., Fallman, M., Gustavsson, J., Stendahl, O., and Andersson, T. (1989) Association of ligand-receptor complexes with actin filaments in human neutrophils: a possible regulatory role for a G-protein. *J. Cell Biol.* **109**, 2791-2899
73. Keefer, J. R., and Limbird, L. E. (1993) The $\alpha_{2A}$-adrenergic receptor is targeted directly to the basolateral membrain domain of Madin-Darby canine kidney cells independent of coupling to pertussis toxin-sensitive GTP-binding proteins. *J. Biol. Chem.* **268**, 11340-11347
74. von Zastrow, M., Link, R., Daunt, D., Barsh, G., and Kobilka, B. (1993) Subtype-specific differences in the intracellular sorting of G protein-coupled receptors. *J. Biol. Chem.* **268**, 763-766
75. Peraldi, S., Nguyen Than Dao, B., Brabet, P., Homburger, V., Rouot, B., Toutant, M., Bouille, C., Assenmacher, I., Bockaert, J., and Gabrion, J. (1989) Apical localization of the alpha subunit of GTP-binding protein $G_o$ in choroidal and ciliated ependymocytes. *J. Neurosci.* **9**, 806-814
76. Lewis, J. M., Woolkalis, M. J., Gerton, G. L., Smith, R. M., Jarett, L., and Manning, D. R. (1991) Subcellular distribution of the alpha subunit(s) of $G_i$: visualization by immunofluorescent and immunogold labeling. *Cell Regul.* **2**, 1097-1113
77. Wang, H. Y., Berrios, M., and Malbon, C. C. (1989) Indirect immunofluorescence localization of beta-adrenergic receptors and G-proteins in human A431 cells. *Biochem. J.* **263**, 519-532
78. Muntz, K. H., Sternweis, P. C., Gilman, A. G., and Mumby, S. M. (1992) Influence of gamma subunit prenylation on association of guanine nucleotide-binding regulatory proteins with membranes. *Mol. Biol. Cell* **3**, 49-61
79. Rimon, G., Hanski, E., Braun, S., and Levitzki, A. (1978) Mode of coupling between hormone receptors and adenylate cyclase elucidated by modulation of membrane fluidity. *Nature (London)* **276**, 394-396
80. Henis, Y. I. (1989) Lateral mobility measurement of cell surface components: applications for molecular pharmacology. *Trends Pharmacol. Sci.* **10**, 95-98
81. Atlas, D., Volsky, D. J., and Levitzki, A. (1980) Lateral mobility of beta-receptors involved in adenylate cyclase activation. *Biochim. Biophys. Acta* **597**, 64-69
82. Jans, D. A., Peters, R., Zsigo, J., and Fahrenholz, F. (1989) The adenylate cyclase-coupled vasopressin $V_2$ receptor is highly laterally mobile in membranes of LLC-$PK_1$ renal epithelial cells at physiological temperature. *EMBO J.* **8**, 2481-2488
83. Kwon, G., Axelrod, D., and Neubig, R. R. (1994) Lateral mobility of tetramethylrhodamine-labelled G protein $\alpha$ and $\beta\gamma$ subunits in NG 108-15 cells. *Cell. Signal* In press
84. Sternweis, P. C. (1986) The purified alpha subunits of $G_o$ and $G_i$ from bovine brain require beta gamma for association with phospholipid vesicles. *J. Biol. Chem.* **261**, 631-637
85. Neer, E. J., Pulsifer, L., and Wolf, L. G. (1988) The amino terminus of G protein alpha subunits is required for interaction with beta gamma. *J. Biol. Chem.* **263**, 8996-8970
86. Remmers, A., and Neubig, R. R. (1993) Resonance energy transfer from fluorescent G protein subunits to membrane lipids. *Biochemistry* **32**, 2409-2414
87. Sargiacomo, M., Sudol, M., Tang, Z.-L., and Lisanti, M. P. (1993) Signal transducing molecules and glycosyl-phosphatidylinositol-linked proteins form a caveolin-rich insoluble complex in MDCK cells. *J. Cell Biol.* **122**, 789-807
88. Raposo, G., Dunia, I., Delvaier-Klutchko, C., Kaveri, S., Strosberg, A. D., and Benedetti, E. L. (1989) Internalization of $\beta$-adrenergic receptor in A431 cells involves non-coated vesicles. *Eur. Cell Biol.* **50**, 340-352
89. Dupree, P., Parton, R. G., Raposo, G., Kurzchalia, T. V., and Simons, K. (1993) Caveolae and sorting in the *trans*-Golgi network of epithelial cells. *EMBO J.* **12**, 1597-1605
90. Fujimoto, T. (1993) Calcium pump of the plasma membrane is localized in caveolae. *J. Cell Biol.* **120**, 1147-1157
91. Fujimoto, T., Nakade, S., Miyawaki, A., Mikoshiba, K., and Ogawa, K. (1992) Localization of inositol 1,4,5-trisphosphate receptor-like protein in plasmalemmal caveolae. *J. Cell Biol.* **119**, 1507-1513
92. Haslam, R. J., Kolde, H. B., and Hemmings, B. A. (1993) Pleckstrin domain homology. *Nature (London)* **363**, 309-310
93. Mayer, B. J., Ren, R., Clark, K. L., and Baltimore, D. (1993) A putative modular domain present in diverse signaling proteins. *Cell* **73**, 629-630
94. Pitcher, J. A., Inglese, J., Higgins, J. B., Arriza, J. L., Casey, P. J., Kim, C., Benovic, J. L., Kwatra, M. M., Caron, M. G., and Lefkowitz, R. J. (1992) Role of beta gamma subunits of G proteins in targeting the beta-adrenergic receptor kinase to membrane-bound receptors. *Science* **257**, 1264-1267
95. Shaw, G. (1993) Identification of novel pleckstrin homology (PH) domains provides a hypothesis for PH domain function. *BBRC* **195**, 1145-1151
96. Musacchio, A., Gibson, T., Rice, P., Thompson, J., and Saraste, M. (1993) The PH domain: a common piece in the structural patchwork of signalling proteins. *TIBS* **18**, 343-348

97. Schurmann, A., Rosenthal, W., Schultz, G., and Joost, H. G. (1992) Characterization of GTP-binding proteins in Golgi-associated membrane vesicles from rat adipocytes. *Biochem. J.* **283,** 795–801
98. Schulze, W., Kossler, A., Hinsch, K. D., Rosenthal, W., Will-Shahab, L., Kuttner, I., Rada, T., Vannauer, M., and Breter, H. (1991) Immunocytochemical localization of G-proteins (alpha subunits) in rat heart tissue. *Eur. Heart J.* **12,**(Suppl. F.) 132–134
99. Hermouet, S., de Mazancourt, P., Spiegel, A. M., Farquhar, M. G., and Wilson, B. S. (1992) High level expression of transfected G protein alpha i3 subunit is required for plasma membrane targeting and adenylyl cyclase inhibition in NIH 3T3 fibroblasts. *FEBS Lett.* **312,** 223–228
100. Stow, J. L., de Almeida, J. B., Narula, N., Holtzman, E. J., Ercolani, L., and Ausiello, D. A. (1991) A heterotrimeric G protein, G alpha i-3, on Golgi membranes regulates the secretion of a heparan sulfate proteoglycan in LLC-PK1 epithelial cells. *J. Cell Biol.* **114,** 1113–1124
101. Ausiello, D. A., Stow, J. L., Cantiello, H. F., de Almeida, J. B., and Benos, D. J. (1992) Purified epithelial $Na^+$ channel complex contains the pertussis toxin-sensitive G alpha i-3 protein. *J. Biol. Chem.* **267,** 4759–4765
102. Brunskill, N., Bastani, B., Hayes, C., Morrissey, J., and Klahr, S. (1991) Localization and polar distribution of several G-protein subunits along nephron segments. *Kidney Int.* **40,** 997–1006
103. Stow, J. L., Sabolic, I., and Brown, D. (1991) Heterogeneous localization of G protein alpha-subunits in rat kidney. *Am. J. Physiol.* **261,** F831–F840
104. Chin, G. J., Vogel, S. S., Elste, A. M., and Schwartz, J. H. (1990) Characterization of synaptophysin and G proteins in synaptic vesicles and plasma membrane of *Aplysia californica*. *Brain Res.* **508,** 265–272
105. Shinohara, H., Kato, K., and Asano, T. (1992) Differential localization of G proteins, $G_i$ and $G_o$, in the olfactory epithelium and the main olfactory bulb of the rat. *Acta Anat. (Basel)* **144,** 167–171
106. Aronin, N., and Difiglia, M. (1992) The subcellular localization of the G-protein $G_i$ alpha in the basal ganglia reveals its potential role in both signal transduction and vesicle trafficking. *J. Neurosci.* **12,** 3435–3444
107. Singer, S., J., Nicolson, G. L. (1972) The fluid mosaic model of the structure of cell membranes. *Science* **175,** 720–731